



PCT/EP 00/0766



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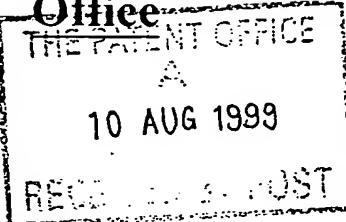
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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

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1. Your Reference	JV/PG3749		
2. Patent application number (The Patent office will fill in this part)	9918745.2		
3. Full name, address and postcode of the or of each applicant (underline all surnames)	GLAXO GROUP LIMITED GLAXO WELLCOME HOUSE BERKELEY AVENUE GREENFORD MIDDLESEX UB6 0NN GB		
Patents ADP number (if you know it)	473587003		
If the applicant is a corporate body, give the country/state of its corporation	GB		
4 Title of the invention	MEDICAL USES		
5 Name of your agent (if you know one)	JANIS F VOLCKMAN (SEE CONTINUATION SHEET)		
"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)	GLAXO WELLCOME PLC GLAXO WELLCOME HOUSE, BERKELEY AVENUE GREENFORD, MIDDLESEX UB6 0NN, GB		
Patents ADP number (if you know it)	7182686001		
6. If you are declaring priority from one or more earlier patent applications, give the country and date of filing of the or of each of these earlier applications and (if you know it) the or each application number	Country	Priority application number (if you know it)	Date of Filing (day / month / year)
7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application		Date of filing (day / month / year)
8. Is a statement of inventorship and of right to grant a patent required in support of this request? (Answer yes if: a) any applicant named in part 3 is not an inventor, or b) there is an inventor who is not named as an applicant, or c) any named applicant is a corporate body.	YES		

See note (d))

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MEDICAL USES

The present invention relates to new uses for EP4 receptor antagonists.

5 The EP4 receptor is a 7-transmembrane receptor and its natural ligand is the prostaglandin PGE₂. PGE₂ also has affinity for the other EP receptors (types EP1, EP2 and EP3).

10 Compounds exhibiting EP4 binding activity have been described in WO98/55468 and EP0855389. GB2330307 describes the use of EP4 antagonists in the treatment of conditions with accelerated bone resorption.

15 It has now been found that EP4 receptor antagonists are of use in the treatment of migraine, neuropathic pain, colon cancer and in increasing the latency of HIV infection.

20 It is believed that selective EP4 receptor antagonists exhibit a number of advantages over current non-steroidal anti-inflammatory (NSAID) and cyclo-oxygenase-2 inhibitor (COX-2i) drugs which act via a number of prostaglandin pathways. By selectively inhibiting the EP4 receptor, the beneficial activities of other prostaglandin pathways are retained. The use according to the instant invention therefore provides greater efficacy and improved gastro-intestinal safety over NSAIDs.

25 The present invention provides the novel use of an EP4 receptor antagonist in the manufacture of a medicament for use in the treatment of migraine, neuropathic pain, colon cancer, and for increasing the latency of HIV infection.

30 In a further aspect the invention provides a novel method of increasing the latency of HIV infection; and for treating migraine, neuropathic pain, and colon cancer; in a mammal, including man, comprising administration of an effective amount of an EP4 receptor antagonist.

heterocyclic group; or amino optionally substituted with protected carboxy or lower alkylsulfonyl,

R² is hydrogen or lower alkyl,

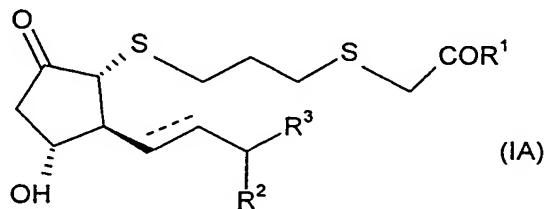
R³ is aryl optionally substituted with halogen,

5 R⁴ is aryl optionally substituted with halogen,

Q is $-A^1 - \begin{array}{c} \text{---} \\ | \\ A^2 \end{array} - A^3 -$ [in which -A¹- is a single bond or lower alkylene,

(A²) is cyclo (C₅-C₉)alkene, cyclo (C₃-C₉)alkane, bicyclo(C₆-C₉)alkene or bicyclo (C₅-C₉)alkane, and -A³- is a single bond or lower alkylene], and X is O, NH or S; which may be prepared according to the methods described therein.

10 Compounds described in EP0855389 are 3,7-dithiaprostanic acid derivatives of the formula (IA):



(wherein R¹ is hydroxy, C1-4alkoxy or a group of the formula:



wherein R⁶ and R⁷, independently, are hydrogen atom or C1-4alkyl,

15 R² is hydrogen atom or hydroxy,

R³ is

(i) C1-8alkyl, C2-8alkenyl or C2-8alkynyl,

(ii) phenyl or C3-7cycloalkyl,

(iii) C1-8alkyl, C2-8alkenyl or C2-8alkynyl substituted by phenyl or C3-7 cycloalkyl,

20 with the proviso that alkyl, alkenyl, alkynyl in (i) or (iii) may be substituted by one hydroxy group, when R² is hydrogen atom;

the symbol --- is a double or single bond;

the formula including the 8-epi equilibrium compound thereof);

suitable buffer is of the following composition: 50mM HEPES, 1mM EDTA, 25 μ g/ml bacitracin, 100 μ M leupeptin, 1mM PMSF, 2 μ M Pepstatin A, pH adjusted to 7.4 with KOH. Following removal of cell debris by a low-speed centrifugation, a pellet of membranes is prepared by a high-speed (48000g) 5 centrifugation of the resulting supernatant. Membrane suspensions such as that described may be stored at -80°C until used.

For assay, membranes expressing human EP₄ receptors are diluted in a pH-buffered medium and mixed with SPA beads coated with a suitable substance to facilitate the adhesion of membranes to the beads. The concentrations of membrane protein and SPA beads chosen should result in SPA binding signal of at least 300 corrected counts per minute (CCPM) when tritiated radioligand at a concentration close to its K_d (affinity value) is combined with the mixture. Non-specific binding (nsb) may be determined by competition between the radiolabelled ligand and a saturating concentration of unlabelled ligand. In order to quantify the affinity of EP4 receptor antagonists, compounds are diluted in a stepwise manner across the wells of a 96-well plate. Radioligand, compound, and unlabelled ligand are then added to a 96-well plate suitable for the measurement of SPA binding signals prior to the addition of bead / membrane mixture to initiate the binding reaction. Equilibrium may be achieved by incubation at room temperature for 120 minutes prior to scintillation counting. 10 The data so generated may be analysed by means of a computerised curve-fitting routine in order to quantify the concentration of compound that displaces 50% of the specific radioligand binding (IC₅₀). The affinity (pK_i) of the compound 15 may be calculated from the IC₅₀ by application of the Cheng-Prusoff correction. Suitable reagents and protocols are: reaction buffer containing 50mM HEPES, 10mM MgCl₂, pH adjusted to 7.4 with KOH; SPA beads coated with wheatgerm 20 agglutinin; 1.25nM [³H]-prostaglandin E₂ as radioligand; 10 μ M prostaglandin E₂ as unlabelled ligand; a three-fold dilution series of compound starting at 10 μ M 25 and ending at 0.3nM is adequate. 30

By application of this technique, 4-(4,9-diethoxy-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetic had a pK_i of 7.00 \pm 0.28 (mean \pm standard deviation of the mean; n = 87).

compositions may conveniently be presented for use in conventional manner in admixture with one or more physiologically acceptable carriers or excipients.

5 While it is possible for the compounds to be administered as the raw chemical, it is preferable to present it as a pharmaceutical formulation. The formulations comprise the compounds together with one or more acceptable carriers or diluents therefor and optionally other therapeutic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

10

The formulations include those suitable for oral, parenteral (including subcutaneous e.g. by injection or by depot tablet, intradermal, intrathecal, intramuscular e.g. by depot and intravenous), rectal and topical (including dermal, buccal and sublingual) administration although the most suitable route 15 may depend upon for example the condition and disorder of the recipient. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the compounds ("active ingredient") with the carrier which constitutes one or more accessory ingredients. In general 20 the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

20

25 Formulations suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets (e.g. chewable tablets in particular for paediatric administration) each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous liquid or a non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, 30 electuary or paste.

30

35 A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant, inert diluent, lubricating,

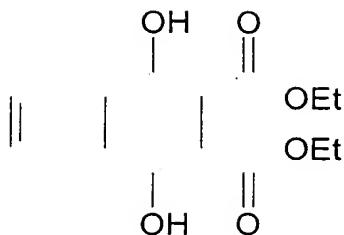
formulation in question, for example those suitable for oral administration may include flavouring agents.

5 The EP4 receptor antagonist compounds for use in the instant invention may be used in combination with other therapeutic agents, for example COX-2i drugs, 5-lipoxygenase inhibitors, low dose aspirin, NSAID's (such as diclofenac, indomethacin), leukotriene receptor antagonists, DMARD's for example TNF inhibitors (such as enbril), methotrexate, adenosine 1 agonists, sodium channel antagonists, and NMDA antagonists such as glycine antagonists. When the
10 compounds are used in combination with other therapeutic agents, the compounds may be administered either sequentially or simultaneously by any convenient route. The invention thus provides, in a further aspect, the use of a combination comprising an EP4 receptor antagonist with a further therapeutic agent in the treatment of migraine, neuropathic pain, colon cancer and in
15 increasing the latency of HIV infection.

20 The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus pharmaceutical formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier or excipient comprise a further aspect of the invention. The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

25 When an EP4 receptor antagonist is used in combination with a second therapeutic agent active against the same disease, the dose of each compound may differ from that when the compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

30 Preferred unit dosage formulations are those containing an effective daily dose, as herein above recited, or an appropriate fraction thereof, of the active ingredient. Conveniently that may be from 5 mg to 1000 mg, such as from 8 mg to 1000 mg, more conveniently 35 mg to 800 mg, and most conveniently 20 to 200 mg, calculated as the free base.

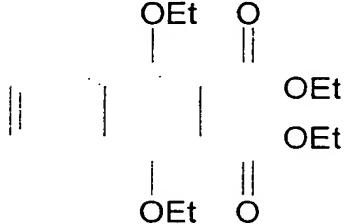


Sodium (60g, 2.6mol) was dissolved in ethanol (1.2L) and the mixture was cooled to 40°C. Diethylphthalate (960ml, 4.83mol) was added and the mixture heated under nitrogen until the temperature reached 115°C. Diethyl succinate (211.3g, 1.21mol) was added dropwise over 45 min. The reaction was heated at 115°C for a further 45 min, cooled to room temperature and poured onto water (1.2L). Ethyl acetate (1L) was added and stirred, the layers were separated and the organics were extracted with sodium hydroxide solution (2N, 1L). The combined aqueous was acidified to pH 3 and the mixture extracted with ethyl acetate (2 x 1L). The combined organics were washed with a saturated solution of sodium hydrogen carbonate (2 x 1.5L), then brine, dried (MgSO_4), filtered and the solvent evaporated under vacuum. The residue was purified using a 2.5kg Biotage column eluting with 5% ethyl acetate / hexane to give ethyl 1,4-dihydroxy- 2,3-naphthalenedicarboxylate as a white solid, (60g, 16%)

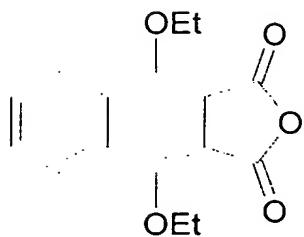
$\delta\text{H CDCl}_3$ 10.44,(2H, s), 8.34,(2H, m), 7.68,(2H, m), 4.37,(4H, q), 1.37,(6H, t).

Intermediate 2

Ethyl 1,4-diethoxy- 2,3-naphthalenedicarboxylate



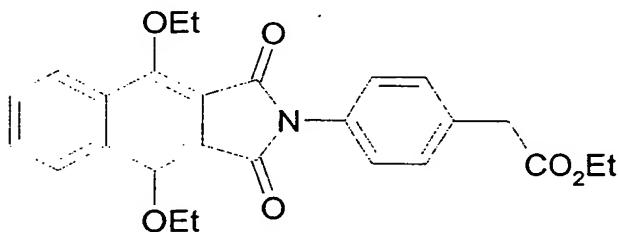
Ethyl 1,4-dihydroxy- 2,3-naphthalenedicarboxylate (30g, 98.6 mmol) and potassium carbonate (150g, 1.09mmol) were stirred in acetone (600ml) under nitrogen. Iodoethane (150g, 0.96mol) was added and the mixture was stirred at reflux overnight. The reaction was cooled, diluted with ethyl acetate and filtered.



1,4-Diethoxy- 2,3-naphthalenedicarboxylic acid (25g, 82mmol) was added to a solution of thionyl chloride (23.3g) in chloroform (150ml) and stirred at reflux for 5 1h. The resulting solution was cooled and evaporated to dryness. Further chloroform was added and evaporation repeated to give 1,4-diethoxy- 2,3-naphthalenedicarboxylic anhydride as a yellow solid (23.3g, 99%).
 δH [$^2\text{H}_6$] – DMSO 8.42,(2H, m), 7.93,(2H, m), 4.53,(4H, q), 1.46,(6H, t).

10 Intermediate 5

Ethyl[4-(4,9-diethoxy-1,3-dioxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetate



15 1,4-Diethoxy- 2,3-naphthalenedicarboxylic anhydride (23.3g, 81.5mmol) and ethyl (4-aminophenyl)acetate (14.8g, 82mmol) were refluxed under nitrogen in acetic acid (160ml) overnight. The mixture was cooled to room temperature and poured into water (1L). The white solid was filtered, washed with water and dissolved in dichloromethane (800ml). The solution was washed with water, brine and dried (MgSO_4) and the solvent evaporated under vacuum to give ethyl [4-(4,9-diethoxy-1,3-dioxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetate as an off-white solid, 33g, 96%.
 δH [$^2\text{H}_6$] – DMSO 8.40,(2H, m), 7.87,(2H, m), 7.42,(4H, s), 4.47,(4H, q), 4.12,(2H, q), 3.76,(2H, s), 1.45,(6H, t), 1.21,(3H, t).

ethanol (146ml) and water (70ml) and heated to reflux for 2h. The solution was cooled to room temperature and the solvent evaporated under vacuum to leave an off-white solid. The solid was slurried in water and the water was evaporated under vacuum. The residue was stirred in hydrochloric acid (2N) for 2h, filtered and washed with water. Drying of the solid at 40° C in a vacuum oven gave [4-(4,9-diethoxy-1-oxo-1,3-dihydro-2H-benzo[f]isoindol-2-yl)phenyl]acetic acid as a white solid (4.5g, 82%)

5 $\delta\text{H} [{}^2\text{H}_6]$ – DMSO 12.27,(1H, b), 8.25,(1H, d), 8.12,(1H, d), 7.86,(2H, d),
7.61,(2H, m), 7.27,(2H, d), 5.10,(2H, s), 4.34,(2H, q), 4.25,(2H, q), 3.54,(2H, s),
10 1.41,(3H, t), 1.37,(3H, t). MS 406, $[\text{MH}^+]$

15 The application of which this description and claims forms part may be used as a basis for priority in respect of any subsequent application. The claims of such subsequent application may be directed to any feature or combination of features described herein. The claims may take the form of product, composition, process or use claims and may include, by way of example, one or more of the following claims.

Abstract

MEDICAL USES

- 5 The present invention relates to the use of an EP4 receptor antagonist in the manufacture of a medicament for use in the treatment of migraine, neuropathic pain, colon cancer, and for increasing the latency of HIV infection.